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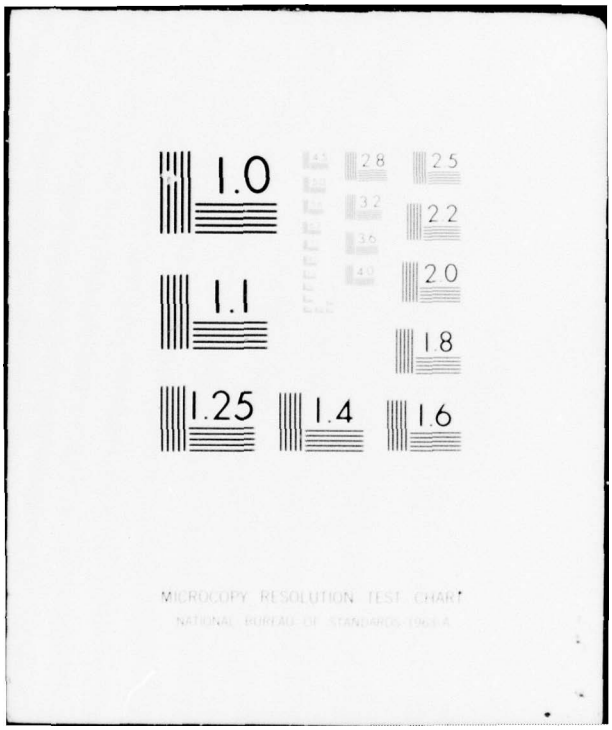
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METABOLIC STUDIES IN MILITARY NUTRITION

FINAL COMPREHENSIVE REPORT

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The Regents of the University of Colorado
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The Surgeon General's Office over a period of years has undertaken an extensive research program into the chemistry, nutrition, and wholesomeness of food preserved by ionizing radiation. As one of the Surgeon General's contractors, the University of Colorado jointly with the U.S. Army Medical Research and Nutrition Laboratory has undertaken the studies of the wholesomeness of food preserved by ionizing radiation. Work on radiated food has been fairly well completed, although a possibility of long term human →

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feeding tests on radiated food still remains. In the meantime, much work on physiological, metabolic, and nutritional research in normal young adult men has been going on making use of volunteer human test subjects. The metabolic ward of USAMRNL has been continuously in operation since 1954. In this endeavor innumerable studies have been carried out and reports of prior studies may be obtained from the Annual Progress Reports.

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Summary of studies done in the Metabolic Division, USAMRNL, Denver, Colorado for the period July 1965 to April 1974 in fulfillment of Army Contract No. DA-49-193-MD-2596 performed under the auspices of the University of Colorado, Boulder, Colorado 80309.

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A. Studies of the Adaptive Responses of Gastrointestinal (G.I.) Tract Enzymes in Animals and Normal Humans

From 1965-1974 this laboratory has carried out extensive studies demonstrating the effects of dietary components, drugs and hormones on the gastrointestinal tract in health and disease. This is a review, in part, the nature and scope of studies which have been done in the past on the Metabolic Ward of the Metabolic Division, USAMRNL. It does not include studies carried out by the Chemistry and Bioenergetics Divisions utilizing the Metabolic Ward.

Diarrhea in humans may result from the absence of jejunal brush border enzymes such as lactase, sucrase and isomaltase (1). Deficiency of these enzymes may occur as part of a generalized process which damages the intestinal mucosa, such as in tropical sprue or celiac disease, or may occur in isolated form in the absence of known precipitating factors (2). Of the disaccharidases, deficiency of lactase is the most common, occurring in a majority of the world's non-white, adult population, and with a lower incidence that increases with age in the white population (3). Lactase may be absent as a congenitqal defect in the infant, or may gradually disappear in adults (1,2,4).

Gentic influence is suggested by the marked differences in the incidence of isolated lactase deficiency in various ethnic populations. Milk consumption varies widely in these groups, and is correlated with the incidence of lactase deficiency. Since the incidence of the adult form increases with age, it has been postulated that lactase deficiency is due to insufficient intake of dietary lactose. However, the role of dietary lactose in the control of human lactase activity has never been demonstrated. It is known that in the rat, sucrase and maltase, but not lactase are increased by dietary carbohydrate (5,6).

These observations raised several interesting questions. Is lactase responsive to dietary lactose? Are jejunal disaccharidases responsive to the dietary disaccharides that are their substrates? And if so, are other intestinal enzymes influenced by diet, and,

if so, what are the dietary factors which control jejunal enzyme activity in man? More generally, what are the reasons for ethnic and individual differences in food consumption, and for food avoidances? Do enzymatic defects other than lactase deficiency lead to the exclusion of certain dietary components which are known to provoke symptoms? Does the continued ingestion of dietary components, which are normally not considered harmful, provoke symptoms when latent gastrointestinal enzymatic or metabolic abnormalities exist? In order to answer these and other questions, we began to study the role of diet in the regulation of jejunal enzyme activity in man.

The effects of dietary sucrose and glucose on intestinal disaccharidase activity were compared in normal volunteer research subjects. In each subject jejunal sucrase and maltase activities were significantly higher on sucrose than on isocaloric glucose diets. The responses to dietary maltose, lactose and galactose were similar to those seen with dietary glucose, i.e., enzyme activity did not increase. However, dietary fructose produced sucrase and maltase responses identical to those seen with dietary sucrose. These studies demonstrated that human jejunal sucrase and maltase activities increased in response to dietary sucrose and fructose, while in contrast, jejunal lactase was uninfluenced by lactose and other dietary sugars. Furthermore, since dietary fructose reproduced the stimulatory effect of sucrose on disaccharidase activity, while glucose did not, fructose appeared to be the active moiety in sucrose. Dietary lactose, galactose, and maltose were similar to glucose in their lack of effect on disaccharidase activity (7).

Following these initial observations, the time required for human jejunal sucrase and maltase activities to adapt to dietary carbohydrate was determined. The increase in jejunal sucrase and maltase activities occurred over 3 to 5 days, and did not further increase thereafter. These activities decreased over 3 to 5 days and then remained unchanged following the institution of a carbohydrate free diet. After the initial 3 to 5 day period, long term

feeding of dietary sucrose did not further influence jejunal disaccharidase activity (8).

The time response of 3 to 5 days coincides with the estimated intestinal cell turnover time in man (9-12). Therefore, we have postulated that the increase in sucrase and maltase activities is due to an effect of the sugar on the crypt cell which becomes apparent as the crypt cells divide and migrate to the tip of the villus over a 3 to 5 day period.

Fasting obese patients have a decrease in disaccharidase activity which returns toward normal when glucose is administered orally or intravenously. In obese patients after fasting, sucrase and maltase activities increase following dietary glucose but do not respond to an isocaloric carbohydrate-free diet (13).

Therefore, studies were performed to compare the effects of isocaloric diets containing varying amounts of specific carbohydrate. Both jejunal sucrase and maltase activities increase progressively in relation to increases in the sugar content of isocaloric diets. At any given level of dietary sugar, sucrose increased enzyme activity to an extent greater than glucose. These studies demonstrated a dose response to dietary sucrose, and to a lesser extent to dietary glucose (14,15). In acute studies, no change in disaccharidase activity was found when isocaloric, constant carbohydrate, high protein and protein-free diets were compared. From these studies it also appears that isocaloric high and low fat diets do not alter disaccharidase activities (15).

These studies confirmed that certain dietary substrates may influence the activity of jejunal enzymes directly involved in their metabolism. Since dietary fructose raised jejunal sucrase activity, it was postulated that other fructose-metabolizing enzymes in the jejunum might be similarly adaptable. Therefore, the effect of dietary fructose, glucose, sucrose, casein, and fasting on selected jejunal glycolytic enzymes in rats and humans was studied with similar results. Fructokinase (FK) and fructose-1-phosphate aldolase (F-1-PA) activities were highest on the fructose diet,

and progressively lower on sucrose, glucose and casein diets, and lowest during fasting. Hexokinase (HK) and glucokinase (GK) activities were highest on the glucose diet, and progressively lower on sucrose, fructose, casein and fasting diets in that order. Fructose-1,6-diphosphate aldolase (FDPA) activity increased equally with all three sugars to a level greater than that seen with the casein diet or fasting (16,17).

In additional studies of glycolytic enzyme activity in rat jejunum and liver, dietary glucose increase both hepatic and jejunal HK, GK, phosphofructokinase (PFK), FDPA, and pyruvate kinase (PK), whereas fructose increased FK, F-1-PA, FDPA and PK (17,18). Human jejunal glycolytic enzyme activities responded to dietary carbohydrate in a similar manner. Fructosediphosphatase (FDPase) activity was highest during fasting and progressively decreased on casein, fructose and glucose diets respectively (19).

Attention was then turned to the time required for human jejunal glycolytic enzymes to adapt to dietary carbohydrate. Following the ingestion of glucose or fructose, the activities of selected glycolytic enzymes increased within 2 hours and reached a maximum at 6 to 12 hours with an increase still present at 24 hours. In contrast to the disaccharidases, the rapid adaptive response of jejunal glycolytic enzymes to changes in dietary sugar suggested a direct effect on the villus epithelial cell (20,21).

Since the preceding studies had shown that specific dietary sugars increased specific glycolytic enzymes, the role of dietary galactose on galactose metabolizing enzymes was investigated in the rat (22) and man (23). The effects of dietary galactose, sucrose, glucose, fructose, casein and fasting upon the activity of four galactose metabolizing enzymes (galactokinase, galactose-1-phosphate uridyl-transferase, uridine diphosphate galactose 4-epimerase, and galactose dehydrogenase) were studied. Galactose produced the greatest increase in the activity of all four enzymes, with decreasing effects from fructose, sucrose, glucose, and casein, respectively. Jejunal disaccharidase activity decreased to a small but significant degree during galactose feeding (7).

It is well known that sex hormones influence glycolytic enzyme activity in hormone-dependent tissues. Therefore, the effects of estrogenic and androgenic compounds on rat jejunal glycolytic enzyme activity were investigated. The oral administration of testosterone, diethylstilbestrol, progesterone, and estradiol-17β increased PFK and PK activities, and decreased FDPase activity. FDPA, HK and GK activities were unchanged. In the male rat, the greatest effect was seen with testosterone, and gradually decreased with estradiol, diethylstilbestrol and progesterone. In the female rat, in contrast, estradiol and diethylstilbestrol had the greatest effect, with lesser effects following testosterone and progesterone. The data demonstrated that the response of jejunal enzymes to sex hormones is determined in part by the sex of the animal, and consists of concomitant stimulation of the glycolytic pathway and depression of the gluconeogenetic pathway irrespective of gender (24). The intramuscular administration of these same hormones failed to influence jejunal glycolytic and gluconeogenetic enzyme activities. Thus, it appears that responsiveness to sex hormones is specifically localized and therefore highly dependent on the route of administration (25).

The dose and time responses of rat jejunal glycolytic enzymes to oral sex hormones were then investigated. A dose response was clearly evident. Statistically significant increases in activity followed the administration of 5 µg of oral testosterone or estradiol, and maximum changes in activity followed 50 µg doses. Minimal changes in activity were detected 2 hours following a single dose of sex hormone, and maximum changes occurred between 8 and 16 hours. These hormonal effects on rat jejunal glycolytic enzyme activity occur a few hours later than the effects of specific sugars (26).

Since both the route and the form of administered steroids appear to influence the responsiveness of jejunal enzymes, we next studied the effects of oral, intramuscular and conjugated sex steroids on jejunal glycolytic enzyme activity in normal and castrated male and female rats. Basal glycolytic enzyme activity

was significantly depressed in the castrated rats of both sexes. Orally administered sex steroids in normal and castrated rats increased jejunal PK and PFK, whereas intramuscular administration of the same steroids increased enzyme activities only in the castrated rats. Conjugated sex steroids (as the glucosiduronide) produced changes similar to the non-conjugated steroids. These resuls are explicable on the basis of a blood-gut barrier. In non-castrate rats, endogenous circulating sex hormones may participate in the formation of a blood-gut barrier only on the serosal side of the jejunum. This would explain why oral sex hormones are effective in non-castrate rats, while intramuscular sex hormones are not. In castrate rats the absence of endogenous sex hormones would impair the formation of the blood-gut barrier, and would thus allow both oral and parenteral sex hormones to be effective (27).

In previous studies, we demonstrated that oral and intramuscular testosterone produced adaptive increases in jejunal PK activity in hypogonadal rats. These rats were maintained on a Purina chow diet which is high in casein. In more recent studies, we demonstrated that the oral testosterone response occurs only in rats which have been fasted or maintained on a high casein diet. The testosterone-repleted normal and castrated rats failed to show an adaptive response of jejunal PK to testosterone when fed a high glucose or high fructose diet. This is in contrast to hypogonadal human males who respond to oral testosterone on a high casein, high glucose or high fructose diet. Interestingly, castration in rats produced a decrease in jejunal PK activity only on the high glucose or high fructose diets. Jejunal PK decreased in hypophysectomized rats, and was partially restored by growth hormone and a combination of other pituitary hormones (unpublished results).

In additional studies, a variety of oral contraceptive agents (especially the estrogen-like agents) produced adaptive changes in PK and adenyl cyclase activity in both normal and castrated female rats. Progesterone and progesterone-like oral contraceptives

had no effect on PK activity, and only one (Provera) caused a decrease in adenyl cyclase activity. When combined estrogen-progesterone agents were administered, adenyl cyclase activity was unaffected but PK increased to values less than with estrogen alone (28). In normal male volunteers, oral testosterone increased the activity of jejunal PK, but had no effect on HK, F-1-PA or FDPA activities (29).

Adrenalectomy in rats caused a marked decrease in both jejunal glycolytic and gluconeogenetic enzyme activities. The responses to different diets (high fructose, glucose, casein) were qualitatively the same in adrenalectomized and normal rats. When corticosterone was given to the adrenalectomized rats, the responses to different diets were also quantitatively similar to those observed in normal rats. Thus, the adrenal gland appears to be necessary for normal qualitative adaptive responses of hepatic but not jejunal glycolytic enzymes to diet (unpublished results). In obese patients undergoing starvation for weight reduction and in normal subjects, oral and intramuscular dexamethasone decreased jejunal glycolytic enzymes (HK, GK, FK, F-1-PA, and FDPA) and increased a gluconeogenetic enzyme (FDPase). This is exactly the opposite to the effects of insulin, tolbutamide, and the sex steroids, which increase glycolytic and decrease gluconeogenetic enzymes (30,31).

Thyroidectomy decreased the activities of rat jejunal PK, when compared to normal non-thyroidectomized rats. The repletion of thyroidectomized rats with thyroxine produced a marked increase in the activities of this enzyme in the jejunum. These changes were noted in fasted rats as well as rats receiving a high casein, a high glucose or a high fructose diet. The addition of thyroxine to normal rats produced a significant increase in PK activity in the fasted rats and those on the high casein or high glucose diets. However, thyroxine had no effect upon PK activity in normal rats on the fructose diet. Changes in PK activities on the different diets were not the same in the liver as in the jejunum. In thyroidectomized rats, the addition of thyroxine increased the activity

of hepatic PK in the fasted group and those receiving the high casein or high glucose diets. However, on the fructose diet, thyroxine had no such effect. In the liver, thyroidectomy and thyroxine had little or no effect upon F-1-PA and FDPA activities, while in the jejunum, thyroidectomy produced a marked increase in F-1-PA activity on each of the diets when compared to the normal non-thyroidectomized animals (unpublished results).

Because of studies in patients with tropical sprue (to be described below), the effect of folic acid on jejunal enzyme activity was investigated in normal volunteer subjects and in obese patients. Oral folic acid had no effect on disaccharidase activity, but significantly increased glycolytic enzyme activity, including HK, GK, FDPA, FK, F-1-PA, PFK, and PK. In addition, it was found that oral folic acid also significantly increased the activity of jejunal FDPase which is a key gluconeogenetic enzyme. The stimulatory effect of folic acid was seen during fasting, and further raised enzyme activities which had already been increased by specific dietary carbohydrate. This stimulation was measurable within 30 minutes and was complete within 24 hours after the administration of oral folic acid, and disappeared within 24 hours after the folic acid was discontinued. Intramuscular folic acid, oral vitamin B_{12}, and tetracycline failed to affect jejunal glycolytic enzyme activity (19,33-35).

In germ-free rats kept on a folic acid deficient diet, jejunal glycolytic enzyme activity dropped to low levels, and gradually returned to normal during folic acid repletion. This occurred at a time when the animals were not anemic, and showed no megaloblastic changes. Folic acid levels were quite low and gradually rose as folic acid was administered (36).

Further studies in folate-deficient rats demonstrated that folic acid was necessary for the adaptive response of jejunal PK activity to oral and intramuscular sex hormones. In these animals, sex hormones did not stimulate jejunal PK activity. In all cases, the activity of each glycolytic enzyme measured was higher on the folate supplemented diet than on the folate deficient diet. No adaptive responses were seen in the normal rats when the sex steroids were administered intramuscularly (37).

Previous studies have demonstrated that an autoclaved folic acid deficient diet produced a marked decrease in the activities of certain jejunal and hepatic glycolytic enzymes when compared with rats fed a folic acid repleted diet. These enzyme changes were indicative of a folate deficient state despite the fact that megaloblastic changes were not observed in the bone marrow. The enzyme changes were restored to normal levels with the addition of folic acid to the diet. Changes in the enzyme levels correlated quite well with the plasma and erythrocyte folate levels. However, the folate levels in the rats receiving the autoclaved folate deficient diet, although markedly depressed when compared with the rats receiving folate in the diet, were considerably higher than those reported in rats receiving sulfathiazole. Thus, a study was designed to compare the effect of an autoclaved folate deficient diet and sulfathiazole on numerous parameters used to assess the folate status of the male rat. These parameters included plasma, red blood cell and liver folate levels, urinary formiminoglutamic acid (FIGLU) levels, bone marrow smears and various jejunal and hepatic glycolytic enzyme activities. In both the autoclaved folate deficient group and the sulfathiazole folate deficient groups, the urinary FIGLU excretion was elevated within two weeks following the initiation of the experimental diets. Throughout the twelve-week study, the urinary FIGLU levels remained elevated and comparable. The urinary FIGLU levels were also statistically significantly higher in the group of rats receiving folate plus sulfathiazole when compared with the group receiving only folate. As noted in the literature and in our previous studies, the folate levels were the lowest in the folate-deficient rats receiving sulfathiazole. However, the enzyme changes were similar in both the autoclaved folate deficient group and the sulfathiazole folate deficient group. These data suggest that both diets are effective in producing the folate deficient state and that sulfathiazole may have some antagonistic role in folate metabolism as demonstrated by the effect on urinary FIGLU levels (unpublished results).

As a result of the preceding studies which demonstrated the adaptive responses of jejunal enzymes to dietary carbohydrate and folic acid, we next investigated the effects of diet, fasting and oral folic acid on the activities of four enzymes involved in folate metabolism: glutamate formiminotransferase (FIT), serine hydroxymethyltransferase (SHMT), methylene tetrahydrofolate dehydrogenase (MTD) and formyltetrahydrofolate synthetase (FTS). Dietary carbohydrate (glucose and fructose) produced significant increases in the activities of three folate-metabolizing enzymes (FIT, SHMT and MTD) when compared to the activities in the casein fed or fasted rats. Only FIT activity showed a significant differential response to the two sugars, with greater activity following fructose feeding. For each of these enzymes, the activities were highest in the carbohydrate fed group, lower in the casein fed group, and lowest in the fasted group. The FTS activity was highest in the casein fed group, lower in the fasted group, and lowest in the carbohydrate fed group. This enzyme did not respond differentially to the different sugar diets (38).

The activities of FIT, SHMT, and MTD were significantly increased following the oral administration of 200 μg of folic acid. The activities of SHMT and MTD were essentially unchanged on both the high and low levels of oral folic acid, while the activity of FTS was unaffected by either level of oral folic acid (38).

Since we had previously determined that jejunal glycolytic enzymes responded to diet and sex hormones and that the folate metabolizing enzymes responded to dietary carbohydrate, we investigated the effects of oral and intramuscular conjugated and unconjugated sex hormones (progesterone, testosterone, and estradiol-17β) on the activities of these four jejunal folate metabolizing enzymes in both normal and castrated male and female rats. These studies demonstrated that oral sex hormones (estradiol in females and testosterone in males) produced significant adaptive increases in the activities of jejunal FIT, SHMT, and MTD in both normal and castrated rats, while intramuscular sex hormones produced no adaptive

changes in the activities of these enzymes in normal rats. The oral administration of conjugated sex hormones also produced adaptive increases in the activities of SHMT and MTD in normal rat jejunum (39).

It had become clear that the activities of several jejunal enzymes were influenced by dietary carbohydrate, folic acid, and sex hormones. However, the mechanism by which increased activity came about remained uncertain. One possibility was that these adaptive changes were the result of de novo protein synthesis. Therefore, to test this hypothesis, studies were carried out to determine the effects of two inhibitors of protein synthesis, actinomycin D and ethionine, on these adaptive changes. Both actinomycin D and ethionine effectively decreased the adaptive responses of jejunal glycolytic and folate-metabolizing enzymes to dietary carbohydrate. Actinomycin D consistently decreased enzyme activities more than ethionine suggesting that the primary mechanism of adaptation to carbohydrate and sex hormones occurred at the level of DNA-dependent RNA synthesis. Since total inhibition of the adaptive responses was not consistently achieved with either of the two inhibitors, other regulatory mechanisms are probably involved as well (40).

We next investigated the in vitro effect of FIGLU on the enzymes which control the alterante pathways of folate metabolism (MTD, FTS, and SHMT). At concentrations as low as 0.05 mM FIGLU markedly inhibited these enzymes in both human and rat liver and jejunum (41).

Histidine, but not other imidazole compounds, markedly increased PK activity in vitro in rat and human jejunal homogenates and in most rat tissues. PK activity decreased in rats on a histidine deficient diet, and returned to normal with histidine repletion. The histidine effect appeared to be an allosteric phenomenon (42).

Since adenosine 3',5'-monophosphate (cyclic AMP) has been demonstrated to mediate hormone action in various tissues and species and since this substance is present in human intestine, it was postulated that cyclic AMP might be involved in the adaptive response of jejunal enzymes to diet and folic acid. Most conditions producing an increase in tissue levels of cyclic AMP do so

by increasing the activity of adenyl cyclase, the enzyme that catalyzes the formation of cyclic AMP from adenosine triphosphate (ATP). Preliminary studies showed that oral estradiol-17β and other estrogen-like oral contraceptive agents produced significant increases in adenyl cyclase activity, but that progesterone and progesterone-like agents were without effect (28). When pharmacologic amounts of nucleotides were administered to rats by mouth, changes were seen in the activities of (cytoplasmic) glycolytic enzymes and in (membrane-bound) adenyl cyclase. Oral cyclic AMP and dibutyryl cyclic AMP increased the activities of the glycolytic enzymes, whereas ATP had an inhibitory effect. Cyclic GMP had a significant effect only on PFK activity (unpublished data). Therefore, adenyl cyclase activity was measured in jejunum from normal males while on various diets and folic acid. Jejunal adenyl cyclase activity was lower during fasting than on various carbohydrate diets. The type of carbohydrate in the diet made no appreciable difference in adenyl cyclase activity which was higher on the high carbohydrate diets than on the high casein diets in both jejunum and liver. Folic acid had no effect on adenyl cyclase activity (unpublished results).

The antimalarial drugs (chloroquine, primaquine, and dapsone) and the antiprotozoal agent, Diodoquin had variable effects on jejunal glycolytic enzyme activity in normal human subjects. (See studies on Acrodermatitis Enteropathica, page 32 .) Since the occurrence of gastrointestinal symptoms with combined chloroquine-primaquine treatment is also variable, it is not surprising that the responses of individual normal human subjects were inconsistent (unpublished results).

Isonicotinic acid hydrazide (INH) has found widespread use in the treatment of tuberculosis because of its efficacy and relative safety. Nevertheless, a small number of patients (about 1%) react adversely to the administration of INH. Gastrointestinal dysfunction including diarrhea is an infrequent problem and is more usually attributed to concomitant para-aminosalicylic acid

(PAS) therapy. A patient who developed diarrhea while on INH treatment and three normal subjects were studied to determine the effect of INH on jejunal glycolytic enzyme activities.

Three normal subjects were treated with INH for one week and with INH plus vitamin B_6 for a second week. INH decreased the activities of jejunal PK and FDPA, increased the activities of FDPase and had no effect on HK. Vitamin B_6 countered the action of INH. Diarrhea did not occur in these subjects when given INH. The individual sensitivity to INH which leads to diarrhea is unclear. (See studies in a patient with presumed pellagra, page 31 .)

Treatment with phenobarbital and ascorbic acid in pharmacologic doses increased the activity of all of the glycolytic enzymes whereas neomycin, an antibiotic which can cause steatorrhea, Dilantin and PAS decreased the activity of all of the glycolytic enzymes (43).

In normal subjects, the activities of HK, GK, FK, F-1-PA, and FDPA fell markedly when ethanol was administered, and returned to control values when ethanol was discontinued. When ethanol and folic acid were administered together, the glycolytic enzyme activities remained at normal levels. Only after the ethanol was discontinued did the expected folic acid-stimulated increase in enzyme activity occur (44,45).

Ethanol has been used for a number of years, both for its nutrient value (7 Kcal/gm) and as an intoxicating agent. Its mechanism of action largely remains unknown. Recent observations have suggested that certain changes in liver triglycerides and glycogen, the urinary excretion of adrenal steroids, and blood glucose and insulin concentrations resulting from chronic or acute ethanol ingestion may also be seen with agents that are known to cause increased tissue cyclic AMP concentrations. This suggested that some of the effects of <u>in vivo</u> ethanol might result from the activation of adenyl cyclase. To test this hypothesis varying amounts of ethanol were added to a tissue preparation containing adenyl cyclase. Assays were performed on human intestine and rat kidney, intestine, liver, fat and brain. Several short chain alcohols were found to stimulate rat intestinal adenyl cyclase

activity with methanol being the most potent. The production of cyclic AMP varied inversely as the number of carbons in the alcohol increased. Other rat tissue adenyl cyclases were stimulated by ethanol but not to the degree seen in intestinal tissue. Human intestinal adenyl cyclase activity was stimulated by ethanol but, maximal activity was obtained only after homogenization of the tissue with the detergent Lubrol WX. These studies suggested that some of the physiologic effects of ethanol may be mediated through its activation of adenyl cyclase and the resultant increase in tissue cyclic AMP concentrations (46).

In acute studies in normal subjects, insulin increased the activities of the jejunal glycolytic enzymes, PK and PFK and decreased the activities of the gluconeogenetic enzyme, FDPase. Glucagon had opposite effects, i.e., the activities of PK and PFK were reduced and FDPase was increased. FDPA was unaffected by either insulin or glucagon. The biopsies were performed 10 minutes after the administration of hormones, thus demonstrating an acute effect of these hormones on jejunal enzymes. Injection of saline produced no effect. In the chronic studies parenteral insulin significantly increased the activities of PK and HK, decreased FDPase and had no effect on FDPA. Oral tolbutamide significantly increased the activities of PK, HK, and FDPA and decreased FDPase. Thus, insulin and tolbutamide had different effects with respect to FDPA which was unaffected by insulin but was increased by tolbutamide and also with respect to HK which increased more with tolbutamide than with insulin (submitted for publication).

In summary, these studies demonstrate that insulin and glucagon are capable of acutely altering jejunal enzyme activities, and are consistent with our previous findings in rat liver. (See work unit 156, The metabolic responses of liver and extra-hepatic tissues to dietary substances, medications and hormones in health and disease.) Tolbutamide was found to have no effect on cyclic AMP concentrations in the jejunum or in the urinary excretion of cyclic AMP. Jejunal enzyme changes were identical to those seen with the administration of insulin.

Clofibrate has been shown to decrease adenyl cyclase activity in rat (47) and human jejunum and fat (unpublished results). Studies were done to determine the effect of this drug on cyclic AMP levels in human tissues and urine, and to determine if its mechanism of action is related to alterations in cyclic AMP metabolism. There was no consistent change in cyclic AMP levels in jejunum and fat. However, in 3 of 4 subjects, following clofibrate cyclic AMP, levels increased in platelets and decreased in the urine. Platelet PK decreased 35% during treatment, while FDPA did not change (unpublished results).

Further studies were carried out to determine the effect of a number of hormones and of cholera toxin on jejunal cyclic AMP. The following substances did not alter cyclic AMP levels in human jejunum *in vitro*: epinephrine, glucagon, insulin, vasopressin, parathormone, serotonin, histamine, acetylcholine, bradykinin, and dopamine. Theophylline, prostaglandin E_1 (PGE_1) and cholera toxin caused a marked increase in cyclic AMP levels (manuscript in preparation).

To date, the question of adaptive change of small bowel mucosal enzymes to a variety of stimuli has been approached *in vivo* using laboratory animals, patients, and volunteer subjects. Now that a substantial body of data has been accumulated with respect to the *in vivo* situation, it is reasonable to determine if *in vitro* experiments would be feasible. Advantages of this approach would include precise control of experimental conditions, convenience, reproducibility and ability to explore mechanisms not testable in the intact animal. A recently described *in vitro* organ culture technique for rabbit small bowel biopsies was utilized (48). The characterization of the system included light microscopic changes during the first 24 hours, stability of disaccharidases, protein determinations, and the effect of folic acid, testosterone, and sugars on the soluble glucose metabolizing enzymes of the small bowel.

The *in-vitro* results parallel the time course and degree of *in-vivo* regulation on these same materials. It was determined that

the technique was applicable and valid to demonstrate enzyme changes in both human and rabbit jejunal biopsies. Exposure of rabbit jejunum to folic acid *in vitro* (50 µg/ml of culture media) resulted in stimulation of enzyme activities and revealed striking correlation with *in vivo* data, i.e., 50 to 100% increases in FDPase, FDPA, and PK activities at 4-6 hours. Cholera toxin (choleragen, 10 mg/ml) caused a significant increase in the concentration of cyclic AMP. There was a typical response to cyclic AMP, i.e., no change in FDPA, a rise in FDPase and fall in PK. This was confirmed by using theophylline in 10^{-3} and 10^{-4} M concentration.

Ethanol, which has been described in this laboratory to be a potent stimulator of adenyl cyclase in lysed cells, was examined as a stimulator of cyclic AMP formation in intact tissue. Ethanol at a concentration of 7 g/dl but not 0.7 g/dl, stimulated cyclic AMP formation which increased in less than 15 minutes and was noted to return to normal at about 4 hours. In contrast to the experiments where cholera toxin stimulated cyclic AMP and then had a reciprocal effect on the gut enzymes studied, ethanol uniformly depressed all enzymes (PK, FDPA, FDPase) regardless of the cyclic AMP levels. Combined stimulation of cultured jejunum with cholera toxin and ethanol revealed both the early increase of cyclic AMP levels noted with ethanol as well as the late changes due to cholera toxin.

B. Studies of the Adaptive Responses of G.I. Tract Enzymes in Selected Patients

As a result of the preceding observations, we hypothesized that failure of the adaptive responses may result from, or lead to gastrointestinal diseases of various types. The data from normal human volunteers were important in order to determine the significance of the results in selected patients.

1. Malabsorptive States. Studies in several patients with jejunal mucosal damage due to tropical sprue and celiac disease revealed low glycolytic enzyme activity and failure to adapt to

dietary carbohydrate. Since folic acid is a specific therapy for tropical sprue, the effect of folic acid on the adaptive responses was tested in such patients. The observation that folic acid alone increased both the glycolytic and gluconeogenetic enzymes led to the extensive studies previously described. Folate therapy restored the normal adaptive response to dietary carbohydrate in patients with sprue, but had no effect on the patients with celiac disease. In one patient with celiac disease the normal adaptive responses were observed only after a gluten free diet had been instituted. A patient with steatorrhea and growth failure due to nodular lymphoid hyperplasia and aggammaglobulinemia had a normal adaptive response to dietary carbohydrate and folic acid (49).

2. Formiminotransferase Deficiency. Formiminotransferase (FIT) deficiency has been described in several Japanese children, all of whom manifested severe mental and physical retardation (50). Two occidental adult patients with FIT deficiency have been discovered and studied in this laboratory. Both have normal to superior intellect. Both complained of intermittent diarrhea, nausea, weakness, fatigue, abdominal cramping, tremor, flushing, tender hepatomegaly (one only), muscle cramps, and pallor, and both complained of dietary intolerance to carbohydrate and protein which aggravated symptoms.

One patient had mild megaloblastic changes in the bone marrow and an elevated serum folic acid. She also had formiminoglutamic aciduria with or without histidine loading. Her gastrointestinal enzymes failed to adapt to dietary carbohydrates or to the usual doses of oral folic acid. Despite being markedly underweight, there was no jejunal histologic abnormality nor malabsorption.

Formiminotransferase deficiency was found to involve the jejunum, red blood cells, and liver. This enzyme converts tetrahydrofolic (THF) acid into the formimino-THF acid using FIGLU as the source of the formimino group. Ultimately the formimino-THF acid is converted into N^{10}-formyl-THF acid which serves as a cofactor in the formation of formylmethionyl-$tRNA_F$, and one of the intermediates in the purine biosynthetic pathway.

This patient has been treated with large amounts of folic acid, Vivonex, dietary restriction, multi-vitamins, and valium (for muscle spasms). The folic acid has increased the activity of her FIT to a small but significant degree, and has increased the activity of her jejunal glycolytic enzymes, although her adaptive responses to dietary substances and folic acid remain poor (51).

Continued treatment of this patient with FIT deficiency with pharmacological doses of folic acid and a carbohydrate free diet was only partially successful. The patient continued to have intermittent diarrhea, profound weakness, severe weight loss, and anorexia, all of which became persistent. Parenteral hyperalimentation was instituted with Aminosol(R) (fibrin hydrolysate) which improved the patient's debilitated state and poor nutrition and increased her weight from 90 to 115 lbs. However, attempts to feed the patient even small amounts orally caused abdominal pain, nausea, and diarrhea. Continued use of Aminosol despite weight gain resulted in hyperglycemia, hepatomegaly due to a fatty infiltration, abdominal pain, and nausea. Use of a synthetic formulated amino acid solution led to resolution of these adverse clinical manifestations. It became apparent that the patient was intolerant to various peptides present in the Aminosol solution. Because of the success with an "elemental" amino-acid solution by parenteral hyperalimentation, oral Vivonex was substituted. With Vivonex all symptoms disappeared, nutrition was greatly improved and body weight was maintained at 120 lbs. The use of single protein test diets showed that the patient was intolerant of beef, pork, tuna fish, soy bean, egg, milk, and peanut protein. These substances caused abdominal pain, hepatomegaly, nausea, diarrhea, and a hepatic fatty infiltrate proven by biopsy. Avoidance of these substances caused resolution of the hepatomegaly and disappearance of the hepatic fatty infiltrate. The patient was able to tolerate chicken, gluten, butter, and various fruits and vegetables. With a judicious mixture of Vivonex and the tolerated foods, the patient has regained her strength, maintained her weight, and has become relatively asymptomatic. It has also been found that she is intolerant of

vegetables belonging to the cruciferae class of plants (cabbage, onion, turnip, radish, etc.). These plants are characterized by a high content of various pungent substances including nitriles. The present hypothesis is that the FIT deficiency has secondarily led to a failure of hepatic detoxification mechanisms which may also account for the patient's intolerance to the usual adult dose of various medications (demerol, codeine, anti-histamines, etc.). This patient with the adult form of FIT deficiency has normal levels of the folate-metabolizing enzymes which are necessary for the alternate pathway of folate metabolism (SHMT and MTD). However, the FIGLU inhibition of the enzymes involved in the alternate pathway of THF utilization which bypasses the FIT reaction could explain why this alternate pathway does not appear to compensate adequately for the FIT deficiency (41).

It has been postulated that because of the deficiency of N^{10}-formyl-THF acid that results from the FIT deficiency the patient may be unable to initiate protein synthesis and also may have a deficiency in purine synthesis. It is not possible to treat the patient with N^{10}-formyl-THF acid since this is a very unstable material. However, it is entirely possible to provide purines in the form of adenine and guanine as dietary supplements. Therefore, studies were designed to investigate the effect of the oral administration of these substances on the activities of jejunal enzymes and the patient's symptoms.

On a controlled liquid diet (Vivonex) providing 1,800 calories per day supplemented with 500 mg of adenine, the patient showed a significant increase in the activities of jejunal PK, FDPA, FDPase, and FIT. This improvement in enzyme adaptation coincided with a decrease in many of the patient's symptoms. However, the absence of folic acid tended to result in the appearance of symptoms as the period of folic acid abstinence was prolonged. Nevertheless, the increase in enzyme activities was substantial. With guanine alone, 500 mg/day, the patient felt worse and enzyme activities were less than control values. Guanine inhibited the stimulatory action of adenine when both substances were given

together. The best response in jejunal enzyme activities was seen when adenine and folic acid were given together. The patient had minimal symptoms with this therapy. These results with the purines were somewhat unexpected since it had been predicted that administration of both purines simultaneously would give the best response if any response at all would be seen. The nature of the guanine inhibition of enzyme activities is not clear. The effect of adenine can be postulated to represent an increase in a deficient purine pool so that an increased amount of purine nucleotides are made available for mRNA synthesis which permits increased protein synthesis, particularly when large amounts of folic acid are present.

Our second patient had G.I. symptoms, food intolerance, FIT deficiency of the jejunum and FIGLU aciduria. With folate treatment, FIGLU aciduria disappeared but food intolerance persisted as did maladaptation. This patient has been treated with pharmacological doses of folate and food restriction. With continued avoidance of offending foods (which are fewer in number than in the first patient), he has remained relatively well. He has been studied less extensively than the first patient, and his problems are therefore less well understood.

3. Maladaptation Syndrome. As a consequence of our experience with the patient with the well-defined syndrome of FIT deficiency in which dietary substances caused diarrhea and failed to cause an increase in jejunal enzyme activity, we investigated the possibility that there might exist a category of patients with gastrointestinal symptoms and an associated failure of adaptation of jejunal enzymes to dietary substances. These patients could not be discovered by the presently used testing procedures since no macroscopic lesion would be present. Such patients could only be found by testing jejunal enzymatic adaptive responses to test diets. As predicted we have identified a large group of patients who lack the normal adaptive responses to diet and/or folic acid. Most of these patients have been considered to have "functional gastrointestinal disease." All had a long history of diarrhea,

and most had a variety of other gastrointestinal complaints. Repeated evaluations including multiple radiographic studies, "routine" laboratory studies, and jejunal mucosal morphology were normal in all.

Most had been treated with a variety of symptomatic regimens with moderate to no control of symptoms. All of these patients were evaluated in the same manner on the metabolic ward. All underwent multiple small bowel biopsies to evaluate glycolytic enzyme activity on a variety of diets and therapeutic regimens. All showed varying degrees of failure of glycolytic enzyme adaptation to diet and/or oral folic acid. In some instances, the patients failed to respond to glucose, while in others there was no response to either glucose or fructose. Most of these individuals also failed to respond to oral folic acid while on a carbohydrate free diet. In most, a high carbohydrate diet provoked obvious worsening of the diarrhea. In a majority, there was improvement with carbohydrate restriction. In some patients, carbohydrate restriction plus folic acid was the most effective. In some of these patients, prolonged carbohydrate restriction has provided continuing freedom from diarrhea, and diminution in other gastrointestinal complaints. In others, symptoms, although decreased in severity and frequency, have continued to occur despite strict dietary adherence. In these patients, there seems to be a clear historical relationship with the ingestion of dietary protein. This suggests that dietary protein as well as dietary carbohydrate may cause symptoms.

Special adjunctive studies were conducted in some of these patients. An in vitro guinea pig ileum preparation was set up in a standard fashion (52). The guinea pig ileum is an extremely sensitive tissue and responds vigorously to low concentrations of a variety of vasoactive and myoactive substances. The response of the systems was standardized with histamine (approx. 1 µg). The response of the pigileum was then measured following addition of a small amount of the homogenate of patient's jejunum obtained at

biopsy. It was found that the jejunal biopsy from two patients contained a material which markedly stimulated the guinea pig ileum. Tissue from other patients and from normal subjects had no effect on the guinea pig ileum preparation.

In two of these patients who were brothers (53), oral glucose produced the characteristic symptoms which mimicked the dumping syndrome. Other patients also had dumping-like symptoms. Since the dumping syndrome which follows gastrointestinal surgery is precipitated by dietary sugar, a common pathophysiologic mechanism is suggested.

These studies demonstrate a gastrointestinal enzyme maladaptation syndrome of a primary nature, and suggest that failure of enzyme adaptation to diet may be responsible for the symptoms in some so-called functional gastrointestinal diseases. They further suggest various possible logical therapeutic approaches for these patients (54,55).

It has been observed repeatedly that some people have a transient period of food intolerance immediately following acute diarrheal episodes. Similar observations have been made in patients who have been fasted for more than a week and in starving individuals upon refeeding. During periods of a low carbohydrate intake or fasting, jejunal disaccharidase activities decrease over a period of 3 days. Jejunal glycolytic enzyme activities decrease within 24 hours on a low carbohydrate intake. Conversely, the same disaccharidases may be increased significantly by giving diets high in fructose. We have found a group of patients with chronic diarrhea who show poor carbohydrate tolerance and an inability to increase their jejunal glycolytic enzymes. The relationship between the poor glycolytic enzyme response and carbohydrate intolerance is not known. If there is a direct relationship between the glycolytic enzyme levels and carbohydrate intolerance, then fasting or a carbohydrate-free diet should decrease jejunal glycolytic enzyme activities to the levels seen in patients with carbohydrate intolerance. There should also be a transient period of poor carbohydrate tolerance similar to that seen in patients whose glycolytic enzymes

cannot adapt normally. To test this hypothesis, four normal male subjects were fed an isocaloric carbohydrate-free diet during one period and a diet consisting of 50% of calories as equal amounts of fructose and glucose. Jejunal disaccharidase activities and glycolytic enzyme activities were measured during both periods. Tolerance to acute loads of sucrose, glucose, and fructose was tested at the end of each dietary period.

There was significant decrease in disaccharidase activities except lactase while on the carbohydrate free diet. Although two of the subjects complained of mild abdominal pain and several loose stools following the sucrose tolerance test, there was no statistically significant difference between the blood sugar and urine sucrose concentrations between the two treatment periods.

There was a significant decrease in glycolytic enzyme activities during the carbohydrate free period. There was a significant increase in blood sugar and plasma insulin levels during the carbohydrate free diet after challenge with both glucose and fructose. The most striking finding was the development of symptoms with both glucose and fructose during the carbohydrate free period. The symptoms consisted of abdominal pain, flushing, dizziness, sweating, nausea, blurred vision and one or two watery stools within 30 to 45 minutes following the sugar load. These symptoms are identical to those described by patients with an inability to increase their glycolytic enzyme activities in response to diet (the G.I. Maladaptation Syndrome).

With reinstitution of the diet with 50% of the calories as equal amounts of glucose and fructose, the glycolytic enzymes returned to control levels. There were no untoward symptoms with glucose and fructose loads and the blood sugar and plasma insulin levels were normal. These studies suggest a cause and effect relationship between human jejunal glycolytic enzymes and gastrointestinal symptoms (56).

4. Hypogonadal States. Previous studies in animals had shown that glycolytic enzyme activity was decreased in castrated animals, and could be increased by repletion with the appropriate hormone. A number of human adult males with hypogonadism due to a variety of causes was studied. Glycolytic enzyme activity was reduced in these patients, and adaptive enzyme changes with dietary manipulation were less than in normal males. Both normal and hypogonadal men showed moderately increased activity of PK after oral testosterone; whereas the hypogonadal men showed moderately increased activity of PK after intramuscular testosterone (29,57).

5. Sucrase-Isomaltase Deficiency. Patients with jejunal sucrase-isomaltase deficiency are usually infants or young children who develop diarrhea and abdominal pain following the ingestion of dietary sucrose or starch. Since sucrose and starch are added to many foods and medications, it is difficult to treat patients with a sucrose-free diet. Since we had shown that dietary fructose increases jejunal sucrase activity, we treated a child with intestinal sucrase-isomaltase deficiency with a high fructose diet. As a measure of therapeutic effectiveness intestinal disaccharidase activities were measured and oral sucrose tolerance tests were performed. During the tolerance tests, urinary and fecal sucrose measurements were also performed to measure the ability of the gut to hydrolyze dietary sucrose. On several synthetic diets which contained an increasing percentage of the total calories as fructose, jejunal sucrase and isomaltase activities rose three-fold over that seen during the fructose-free period. There was also concomitant improvement in the oral sucrose tolerance test. Symptoms decreased along with the amount of sucrose excreted in the urine, and stool and blood glucose levels rose. Appropriate testing failed to reveal the presence of a sucrase inhibitor in the patient's tissue. Following the study, the patient was returned to an ad libitum sucrose deficient diet in which fructose was the only carbohydrate. She has done well throughout the period of follow-up, despite the occasional intake of dietary sucrose. These studies suggest that the patient is producing an enzyme which

is only partially active. Fructose, by increasing the amount of enzyme protein synthesized, and therefore the amount of active enzyme, can alleviate the symptoms formerly caused by small amounts of dietary sucrose (58).

6. Dermatitis Herpetiformis. It has been found that patients with dermatitis herpetiformis may or may not have small intestinal lesions that resemble those seen in celiac disease (gluten enteropathy). Treatment of such patients with a gluten-free diet has given variable responses: no change in the skin lesions, clearing of skin lesions on lower dosages of medication, and clearing of skin lesions without additional therapy. Some, but not all, of the patients with jejunal lesions have steatorrhea. Only some of the patients with steatorrhea respond to a gluten-free diet. We investigated three patients with dermatitis herpetiformis to determine if gastrointestinal lesions were present and if jejunal glycolytic enzymes responded to diet or folic acid.

None of the patients had gastrointestinal symptoms. One had normal jejunal histology and normal glycolytic enzyme adaptive responses. A gluten-free diet was tried empirically, but it seemed to have little effect on her skin lesions. The other two patients had abnormal jejunal histology and abnormal glycolytic enzyme adaptive responses. A trial of a gluten-free diet in them failed to affect the skin lesions, the jejunal histology or the glycolytic enzyme adaptive responses. The nature of the jejunal lesions in dermatitis herpetiformis and the relationship to the skin lesions remain unknown.

7. Riboflavin Deficiency. An assay for flavokinase utilizing ^{14}C-riboflavin has been developed in our laboratory. In conjunction with a vitamin B_2 deficiency study utilizing normal subjects, measurements of jejunal flavokinase were performed to determine the effect of riboflavin deficiency on this enzyme.

The flavokinase activity of jejunal tissue of six normal subjects on two levels of dietary protein were measured during a control period, after eight weeks of deficiency and after two weeks of refeeding with riboflavin. The activity of the enzyme did not

change through these periods. These results are different from those found in the rat liver where a decrease in the enzyme activity occurs. In human red cells, the activity is increased during the deficient period. These results suggest that flavokinase responds differently to riboflavin deficiency in different species.

8. Studies in a Patient with Presumed Pellagra. Investigations were carried out in a patient with pellagra associated with alcoholism. This patient was a 41-year old Negro man who developed a rash on his forearms, wrists, hands, back, head, and face while stationed in Vietnam in May 1972. He was hospitalized at Fitzsimons Army Medical Center (FAMC) in the summer of 1972, where he was treated for a photoallergy. Improvement occurred although the studies were not consistent with the diagnosis. After discharge from FAMC, he resumed his alcohol intake and poor eating habits and recurrence of his skin eruption on sunlight-exposed areas precipitated rehospitalization in October 1972. It was realized that his skin eruption was consistent with the diagnosis of pellagra. The patient was then studied on the Metabolic ward to determine the effect of gradual vitamin supplementation on jejunal enzyme activity. Although we cannot absolutely exclude borderline or mild deficiency of other vitamins and nutrients, physical examination revealed no evidence of other deficiency. Biochemical assessment of his nutritional state showed normal erythrocyte transketolase, erythrocyte transaminase, and erythrocyte glutathione reductase (FAD stimulation) consistent with a normal state of vitamin B_1, B_6, and riboflavin nutriture. Plasma vitamin C was 0.45 mg/100 ml. Urine for niacin was subnormal.

On a low protein diet without vitamin therapy, the patient's jejunal enzyme activities (PK, FDPA, HK, and FDPase) were low. With supplementation of all vitamins except nicotinic acid there was a dramatic rise in all enzyme activities. PK increased from 75.4 nmoles/min/mg protein to 98.6 in four days and 142.6 after three days of folic acid, 15 mg/day. With the addition of niacin 50 mg b.i.d., PK rose in three days to 175.4 and to 252.2 with folate supplementation plus niacin for two days. Similar rises

were seen in the other enzyme activities. The patient received a 2,500 calorie diet containing 25 gms of casein throughout the study. On this basic diet, it was estimated that his total daily supply of niacin from the diet was 6.2 mg. Folic acid 400 µg/day was provided with the general vitamin supplementation.

A vitamin deficient diet, especially one deficient in niacin, (even though the individual has no clinical evidence of generalized vitamin deficiency) leads to a marked decrease in jejunal enzyme activities. Repletion with vitamins quickly increases enzyme activities, even though nicotinic acid may be deficient. Repletion thereafter with nicotinic acid rapidly increases jejunal enzyme activities to still higher levels. The responsiveness of jejunal enzymes to folic acid is enhanced by nicotinic acid. The reasons for the absence of diarrhea in this patient are unclear, as is the mechanism of diarrhea in patients with pellagra. (See studies in normal subjects with INH, page 18.)

9. Acrodermatitis Enteropathica. Acrodermatitis enteropathica (AE) is a rare and puzzling disorder of childhood characterized by severe diarrhea and typical skin lesions involving the body orifices and distal extremities. The disease is frequently fatal during infancy or childhood. Some years ago, Diodoquin(R) was found empirically to be an effective form of therapy. The pathophysiology of this disease and the reasons for the efficacy of Diodoquin(R) remain unclear. An 18-year old female patient with a life-long history of AE has been studied. The patient was one of the first to receive Diodoquin(R) therapy, and is one of the oldest known living patients with this disorder. Occasional attempts at discontinuing Diodoquin(R) have resulted in recurrence of her dermatitis. Continued therapy has controlled her symptoms and allowed her to enjoy a normal mode of living. Systematic studies in AE have rarely been performed since the afflicted individuals have usually been children. This patient presented the unique opportunity to study the disease in an adult.

She underwent an extensive evaluation while on and off Diodoquin(R) therapy, in an attempt to evaluate the underlying pathophysiology and possibly determine the role of Diodoquin(R). Previous investigators suggested that tryptophan metabolism was abnormal in this disorder. However, the excretion of xanthurenic acid and other tryptophan metabolic intermediates after tryptophan loading while the patient was on and off Diodoquin(R) therapy was normal and thus failed to confirm this hypothesis. The adaptive responses of this patient's jejunal glycolytic enzymes to dietary carbohydrate and folic acid were normal and did not seem to show any changes with regard to the Diodoquin(R) treatment (see page 17). An abnormal glucose tolerance test of the diabetic type was found on several occasions, and was not related to the Diodoquin(R) treatment. The significance of this finding is uncertain, although one grandparent had a history of diabetes mellitus. Hypolipidemia (with decreased cholesterol, free fatty acids, and triglycerides) was also consistently found. An abnormal lipoprotein phenotype (type IV) secondary to diminished beta and pre-beta lipoproteins was also found on several occasions. Subsequent studies of unsaturated fatty acids revealed a decreased level of arachidic (20:0) and markedly increased levels of arachidonic (20:4) acid. Such fatty acid abnormalities had been described in two other patients with this disorder (59).

The defective synthesis and/or release of essential fatty acids has been thought to play an important pathogenic role in this disorder, although the exact mechanism has not been clarified. Recent studies have shown that fatty acids are converted directly or indirectly into prostaglandins, and the fatty acid abnormality seen in our patient suggests that co-existent abnormalities of prostaglandin metabolism may also be present. Since prostaglandins are present in significant concentrations in both skin and gut which are the target organs in AE, these results suggest that an underlying abnormality of prostaglandin metabolism may be involved in the disease. The abnormalities in lipid and carbohydrate metabolism are less well understood but may be related to the defects in fatty

acid metabolism. Whether those abnormalities are related to defects in prostaglandin metabolism or to other metabolic defects remains uncertain.

It is also known that certain of the lipid classes are highly reactive and especially subject to peroxidation. These lipid peroxides are toxic, and could lead to cellular functional and structural defects, inflammatory changes, and potentially overt clinical manifestations. The metabolism of fatty acids is complex and incompletely understood. There are, however, cellular mechanisms which operate to protect against peroxide formation. One such enzymatic mechanism is glutathione peroxidase, which serves to trap and destroy a variety of peroxides, including hydrogen peroxide and unsaturated fatty acid peroxides. Interestingly, this enzyme is present in skin and gut, but its exact function in these two organs remains unclear. It is postulated that a defect in glutathione peroxidase could lead to fatty acid abnormalities since dysfunction in both skin and gut are the characteristics of AE. It is of interest that levels of glutathione peroxidase activity are related to dietary selenium intake.

It has recently been shown that oral zinc sulfate is therapeutically effective in patients with AE. The role of zinc in this disorder is not known. Glutathione peroxidase is a selenium-requiring enzyme and it is postulated that zinc, which is also a divalent cation, may be substituting in some manner for the selenium.

10. Total Parenteral Nutrition. Total parenteral nutrition (TPN) is a valuable and often life-saving means of supplying adequate nutrients to patients unable to absorb adequate oral nutrition. Since the intestinal tract is by-passed by this procedure, we speculated that the apparent adequate nutrition was not sufficient to maintain the intestinal tract function to the same degree as with oral nutrients. Thus, biopsies were taken before, during, and after TPN in three infants with intractable diarrhea of undetermined etiology, and assays of jejunal enzyme activities were performed.

All patients showed abnormal intestinal morphology with blunted villi, dilated lymphatics, and inflammatory infiltrate. Disaccharidase activities were deficient in all patients before TPN. After

14 to 16 days of TPN, all patients showed a return toward a normal morphologic pattern and near normal disaccharidase activities. After complete oral alimentation for a period of one week, there was no further morphologic improvement but the sucrase and maltase activities increased significantly. These findings demonstrated that TPN will allow a return to a more normal morphologic pattern but that without the presence of luminal nutrients the intestinal disaccharidases (sucrase and maltase) do not have as high an activity as will occur with oral nutrients. This may cause deficient disaccharide hydrolysis with resultant malabsorption of disaccharides which occurred when the infants were started on oral feedings. These findings have led to more appropriate management of patients with intractable diarrhea as well as to the management of patients receiving TPN who must be converted to oral feedings (56).

These early results indicated that some patients with intractable diarrhea might be more able to absorb elemental nutrients (e.g., Vivonex) since both the absorptive area as well as certain digestive enzymes are secondarily deficient. This would obviate the use of TPN and its inherent complications. When patients are converted from parenteral to oral feedings, a gradual increase in concentration and volume of the nutrients should be given over a period of 3 to 5 days to allow time for adequate adaptation of intestinal enzymes. This practice has proven very beneficial in a number of patients who, from past experience, might have been expected to develop a transient period of malabsorption with the reintroduction of oral feedings.

Since a relatively large number of patients with chronic illnesses become malnourished for a variety of reasons, similar types of studies should be performed in patients who require parenteral nutrition as well as those who are able to tolerate oral feedings with the elemental diets. Information obtained from these studies should lead to a more rational approach to the possible etiology and treatment of some infants who appear to have a new type of

syndrome which is manifested by total food intolerance and who can be nourished only by prolonged parenteral nutrition. These studies also are applicable to understanding many adult onset diarrheal syndromes and may lead to a more rational therapeutic approach in these patients.

11. Studies in Hypothyroid Patients. Because our studies in animals showed an effect of thyroid hormone on glycolytic enzyme activities in both normal and thyroidectomized and hypophysectomized rats, we extended these studies to humans. We have been particularly interested in the relationship between glycolytic enzyme activities in human jejunum and carbohydrate tolerance. Hypothyroidism is characterized by carbohydrate intolerance and there is some evidence that this state is accompanied by insulin resistance. It appeared appropriate to test carbohydrate tolerance in hypothyroid patients and to relate this to jejunal glycolytic enzyme adaptation before and after replacement with thyroid hormone. Two patients have been studied to date.

Distinct abnormalities in jejunal glycolytic enzyme activities were noted in the one patient in whom studies have been completed. Levels of jejunal PK activity were low during fasting and were even lower during administration of glucose and fructose. Administration of cytomel (T_3) produced a rise in PK activity and restored the adaptation to glucose feeding. Abnormalities were also noted in FDPase adaptation (manuscript in preparation).

In summary, we have performed extensive studies over the past several years which have clearly demonstrated the influence of dietary carbohydrate, folic acid, drugs, and various hormones on jejunal enzyme activity. These studies have provided important information regarding the mechanisms of gut enzyme regulation, and have led to the identification of a new category of gastrointestinal disease (the G.I. Maladaptation Syndrome). Furthermore, these studies have provided a basis for the rational therapy of several hereditary enzymatic defects.

These studies have established the important principle that gastrointestinal enzymes adapt to dietary substances, and that failure of this adaptive response can lead to disease. The adaptive principle can be utilized not only to investigate gastrointestinal disease which is inaccessible to other types of clinical evaluation, but also as an innovative and novel therapeutic approach.

C. Studies of the Adaptive Responses of Hepatic Enzymes in Animals and Normal Humans

In the past several years, we have studied the normal adaptive responses of certain hepatic enzymes to different dietary stimuli in the rat. Specific sugars were found to have specific effects on certain hepatic enzyme activities. A high fructose diet when compared to a high glucose diet increased the activities of the enzymes specifically involved in fructose metabolism {fructokinase (FK), fructose-1-phosphate aldolase (f-1-PA} as well as other key rate-limiting enzymes of glycolysis {pyruvate kinase (PK) and phosphofructokinase (PFK)} (16,18). In contrast, a high glucose diet compared to a non-carbohydrate diet produced an adaptive increase in two enzyme activities unique to glucose metabolism {hexokinase (HK) and glucokinase (GK)} as well as other key rate-limiting enzymes of glycolysis, PK and PFK (16,18). The glucose and fructose effects can be called a "specific carbohydrate effect" which is distinct from a "generalized caloric effect" and are observed by comparing the glycolytic enzyme activities in fasted rats with those receiving a high protein, carbohydrate-free diet. The high protein diet adaptively increased all of the glycolytic enzyme activities significantly above the levels seen in the fasted rats {HK, GK, PFK, PK, F-1-PA, FK, and fructosediphosphate aldolase (FDPA)} (16,18).

In contrast to the glycolytic enzymes, the activities of the four rate-limiting enzymes of gluconeogenesis {pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructosediphosphatase (FDPase), and glucose-6-phosphatase (G-6-Pase)} were

highest in fasted rats and decreased, in order, on high protein, high fructose, and high glucose diets, respectively (18,61-64). The marked elevation in activities of the four key gluconeogenetic enzymes during fasting and on a high protein diet corresponded to the enhanced gluconeogenesis seen on these same diets (61-64).

Oral folic acid produced a generalized adaptive increase in all of the glycolytic enzyme activities measured to date (FK, HK, GK, F-1-PA, FDPA, and PK) as well as one gluconeogenetic enzyme, FDPase (36). Interestingly, PEPCK was the only cytoplasmic enzyme activity measured to date which was unaffected by oral folic acid (65).

Since dietary carbohydrate produced marked adaptive increases in the activities of different carbohydrate metabolizing enzymes, we studied the effects of oral folate on one hepatic folate-metabolizing enzyme, glutamate formiminotransferase (FIT). When 2400 µg of oral folate was administered for two consecutive days to normal male rats, there was a significant increase in hepatic FIT activity (65).

In addition to dietary factors and oral folate, numerous hormones produced adaptive changes in hepatic glycolytic and gluconeogenetic enzyme activities. Intravenous glucagon, insulin, and epinephrine produced rapid changes (within minutes) in certain hepatic enzyme activities, while intraperitoneal corticosterone and thyroid hormone elicited a slower response (within hours).

Intravenous glucagon and epinephrine produced a rapid increase in hepatic FDPase activity and rapid decrease in PK and PFK activities, while insulin produced reciprocal effects on these same hepatic enzymes (66-71). The hormone-induced changes appeared to be specific since none of these hormones exerted any effect on FDPA activity. Pretreatment of the rats with two inhibitors of protein synthesis (actinomycin D and puromycin) failed to block the adaptive changes with the hormones suggesting that the mechanism does not involve de novo protein synthesis. The acute responses of hepatic PK, GK, and FDPase activities have been demonstrated in both the rat and man (72).

Intravenous glucagon, insulin, and epinephrine also produced rapid changes (within minutes) in hepatic FIT activity in the rat (73). Limited evidence in human patients demonstrated that similar effects also occurred in man (73). Intravenous glucagon and insulin produced a rapid increase and decrease, respectively, in hepatic FIT activity which was unaltered by pretreatment of the rats with either actinomycin D or puromycin. Intravenous epinephrine also produced a rapid increase in hepatic FIT activity (73). The stimulatory effects of glucagon and epinephrine on hepatic FIT and FDPase activities were mimicked by intravenous cyclic adenosine 3-,5'-monophosphate (cyclic AMP) suggesting that these hormonal responses may be mediated through increased cyclic AMP production (67,69,73). Our suggestion is that the rapid insulin, glucagon, and epinephrine effects on hepatic FIT, PK, PFK, and FDPase activities may be due to dephosphorylation-phosphorylation mechanisms analogous to those involved in the regulation of glycogen metabolism (74).

Other investigators using independent approaches have adduced evidence that is consistent with this hypothesis (75-79). In recent years, it has been shown that the activity of key enzymes of different metabolic pathways are altered by phosphorylation. These include the hormone-sensitive lipase in adipose tissue (80), PK (81), acetyl CoA carboxylase (82), and pyruvate dehydrogenase (83).

In contrast to the rapid specific effects of intravenous insulin, glucagon and epinephrine, intraperitoneal thyroxine and corticosterone exerted a slower permissive effect upon hepatic PK, FDPA, and FDPase activities. Adrenalectomy significantly decreased the activities of these enzymes while corticosterone replacement returned the activities toward or above normal levels (84). Thyroidectomy depressed only hepatic FDPase activity, while replacement with thyroxine played a permissive role in regulating hepatic PK, F-1-PA, and FDPase activities (85).

D. Studies of the Adaptive Response of Hepatic Enzymes in Selected Patients

Because of the known stimulatory effect of pharmacologic doses of oral folic acid on jejunal glycolytic enzyme and FDPase activities in man (33, 19), we speculated that human hepatic glycolytic enzymes and FDPase activities might be affected in a similar manner. If this were so, then pharmacologic doses of folate might be useful in the treatment of patients with a deficiency of F-1-PA [hereditary fructose intolerance (HFI)] or a deficiency of FDPase (which causes hypoglycemia). Thus, we studied the effect of oral folate in one patient with a genetic deficiency of F-1-PA and four patients with FDPase deficiency.

In a child with HFI, oral folate increased F-1-PA activity 70%, although it remained below the normal range (86). This small increase in activity might have permitted an increased metabolism of fructose. After three months of folate treatment, the patient's liver size decreased to normal and the hepatic fatty infiltrate present at the beginning of the study completely resolved (86). Variable improvement occurred when the patient was on a "fructose free diet" without folate, but on the same diet plus folate there was marked symptomatic, histologic, and biochemical improvement. The patient has remained asymptomatic for over a year and gained weight and increased in linear growth appropriately while receiving continuous folate treatment. Because the patient developed shock during a fructose tolerance test during the initial diagnostic studies prior to referral, a fructose tolerance test was not repeated. Therefore, we cannot say unequivocally that the folate therapy has made the patient more resistant to an acute challenge of fructose.

A child with ketotic hypoglycemia due to hepatic and jejunal FDPase deficiency showed an increase in hepatic FDPase activity to 75% of the normal range following oral folate therapy (87). This increase in activity apparently was sufficient to cause a normal response to the ketogenic diet after folate treatment. The ability to convert glycerol to glucose was not improved, but after a glycerol

load,blood glucose concentrations decreased less and clinical symptoms did not occur. Similar changes were seen with fructose and alanine tolerance tests.

Another patient with persistent hypoglycemia (15-20 mg/dl) also had deficient hepatic levels of FDPase activity which were increased about 50% following pharmacologic oral folate therapy (88). Following oral folate treatment, there was a marked improvement in blood glucose to 40 mg/dl. The patient also showed an improved ability to convert glycerol to glucose following continuous folate treatment. The same effect was observed with a fructose tolerance test before and after folate.

Fasting and reactive hypoglycemia associated with low jejunal and hepatic FDPase activity were found in a young mother and her 19-month old daughter (89). No other cause for the hypoglycemia was found. Large doses of oral folic acid increased hepatic and jejunal FDPase activity in both patients. Concomitantly with folic acid therapy, there was subjective improvement in the adult's symptoms and objective improvement in the ability of the child to maintain normal plasma glucose levels.

These observations in one patient with HFI (86) and four patients with hypoglycemia due to an FDPase deficiency (87-89) demonstrated that the folate-induced increase in jejunal enzymes also occurred in liver. Furthermore, it appears that certain hepatic enzyme deficiency diseases may show clinical and biochemical improvement with pharmacologic doses of folic acid. Other investigators (90) have demonstrated that oral folate enhanced gluconeogenesis in a child with FDPase deficiency following fasting and a glycerol tolerance test.

Because of the known antagonisms between alcohol and folate (91-93), we studied the effect of alcohol on jejunal glycolytic and hepatic enzyme activities in normal human volunteers and rats. We demonstrated that oral ethanol ingestion in man caused a decreased activity of jejunal enzymes which was only partially reversed by oral folate (44,45). The administration of oral ethanol with folate failed to produce the normal stimulation of enzyme activities seen

with oral folate alone (33,44). In rat experiments, we have demonstrated a similar antagonism of oral ethanol to folate on hepatic enzyme activities (65,94). Specifically, oral administration of ethanol significantly decreased the activities of two key gluconeogenetic enzymes (PC and FDPase), one glycolytic enzyme FDPA and one folate-metabolizing enzyme (FIT). In each instance, the administration of 2400 µg daily of oral folate in conjunction with the ethanol prevented these alterations in hepatic enzyme activities (65,94). Our data augment the knowledge that ethanol and folate are antagonistic and suggest that oral folate might offer a protective effect against hypoglycemia in rats receiving alcohol.

Chronic ingestion of excessive alcohol is often associated with cirrhosis and malnutrition (95). Preliminary evidence in patients with alcoholic cirrhosis demonstrated a uniform depression in hepatic FDPase activity, while the activities of hepatic PK and FDPA were normal. If the depression in FDPase activity in the cirrhotic liver was a primary cause of impaired gluconeogenesis (as in non-cirrhotic FDPase deficiency), then this observation may offer a possible explanation of alcohol-induced hypoglycemia and perhaps the mechanism of alcohol-induced hepatic injury. In this connection, it is worth noting the observation of Henley et al. (96) who showed impairment of gluconeogenesis in the experimentally produced cirrhotic rat.

Ethanol in increasing concentrations caused a proportional rise in adenyl cyclase activity in the rat liver as well as in other tissues. The rat liver enzyme showed maximal alcohol stimulation only after treatment with Lubrol WX. When a combination of ethanol and fluoride was added to the incubation medium, there was an additive stimulatory effect on the adenyl cyclase activity (46).

Two children with G-6-Pase deficiency, massive hepatomegaly, lactic acidosis, hyperuricemia, hyperlipidemia, and growth retardation have been studied. Clofibrate administration and total parenteral nutrition (TPN) caused a marked improvement in both patients. In one patient with G-6-Pase deficiency, the administration of clofibrate

for one week decreased hepatic FDPA activity, increased FDPase activity and had no effect on PK activity. The prolonged administration of clofibrate to rats resulted in similar changes in hepatic enzyme activities with the exception that PK activity was also significantly lowered. On the basis of the presumed mechanism of action of clofibrate and TPN, it was postulated that chronic hypoglycemia caused increased output of pancreatic glucagon which secondarily increased hepatic cyclic AMP concentrations which in turn affected the rate of glycolysis, glycogenolysis, gluconeogenesis, and lipolysis (97).

In the absence of G-6-Pase the normal compensatory mechanisms cannot increase the hepatic output of glucose in the patients as compared to normal subjects. Therefore, there is a continuous output of glucagon which causes the secondary manifestations of the disease. Since both clofibrate and the constant glucose infusion inhibit adenyl cyclase and glucagon output respectively, a diversion of pancreatic blood flow and hence glucagon away from the liver might also be effective in the therapy of this disease. Furthermore, insulin would also be diverted to peripheral tissues and might increase the growth patterns in these markedly growth-retarded patients (97).

Porta-caval shunts (Eck fistulas) were performed (by Dr. T. Starzl, University of Colorado Medical Center) in these patients who have been followed now for ten months. During this period, their height increased by 3/4 to 1 cm/month, and serum triglyceride, lactate, uric acid, and platelet function became normal. Peripheral blood insulin levels and growth hormone levels increased dramatically. The hepatic size decreased by 20% and the fatty infiltrate that had been present decreased by 20% (as measured in terms of mg triglyceride/gram tissue). Although both patients have continued to have occasional hypoglycemic episodes, they are markedly improved (97).

The inability of these patients to maintain proper blood glucose levels sets in motion hepatic gluconeogenetic, glycogenolytic, and lipogenic mechanisms which results in rapid glycogen re-synthesis, fatty infiltration of the liver and the other secondary complications of the disease. Treatment to interrupt this sequence of

events was of benefit in preventing the secondary manifestations induced by the hypoglycemia but frequent feedings are still required to maintain blood glucose values near the lower limit of normal.

Our significant accomplishments during the period 1965 through 1975 are the following:

1. The jejunal disaccharidases, sucrase and maltase, increase in activity to dietary sucrose and fructose but jejunal lactase does not respond to dietary lactose or galactose.

2. Jejunal glycolytic enzymes increase in activity to dietary sugars.

3. The disaccharidase response takes 3 to 5 days, while the glycolytic enzyme response takes 6 to 12 hours.

4. Utilizing the disaccharidase adaptive responses we were able to treat a patient with sucrase-isomaltase deficiency with dietary fructose.

5. Oral folic acid but not intramuscular folic acid increases jejunal enzyme activities.

6. Two adult patients with formiminotransferase deficiency were discovered and treated with a carbohydrate restricted diet and folic acid.

7. Because of the connection between failure of jejunal enzymes to adapt to carbohydrate in the patients with formimino-transferase deficiency we studied patients with diarrhea of unknown cause and found some whose jejunal enzymes did not adapt to dietary carbohydrate. Many of these patients have been treated successfully with carbohydrate restricted diets and oral folic acid.

8. Folic and ethanol are antagonistic to one another. Alcohol decreases jejunal glycolytic enzyme activities, particularly fructos-ediphosphatase, and folic acid reverses this effect. Ethanol activates jejunal adenyl cyclase.

9. The effects of numerous drugs on jejunal glycolytic enzyme adaptation have been studied. Enzyme activity is increased by phenobarbital, tolbutamide, and ascorbic acid in pharmacologic doses,

decreased by neomycin, Dilantin, PAS, and ethanol, inconsistently changed by INH and various antimalarial agents, and unaffected by tetracylcine and vitamin B_{12}.

10. Jejunal glycolytic and gluconeogenetic enzymes respond to various hormones. The sex steroids have specific effects on enzyme activity depending on the sex, hormonal, and nutritional status of the animal, and the form and route of administration. The enzyme effects of adrenal steroids are exactly the opposite to those of insulin and the sex steroids. Pituitary and thyroid influence on enzyme activity has also been demonstrated.

11. One patient with acrodermatitis enteropathica has been studied. A normal gastrointestinal adaptive pattern was found. No defect in tryptophan metabolism could be uncovered. A generalized hypolipidemia was consistently present along with a lipoprotein electrophoretic pattern of hypobetalipoproteinemia. Serum arachidonic acid levels were consistently low. The efficacy of diodoquin treatment remains unexplained, as does the apparent curative effect of oral zinc sulfate.

12. Dietary sugars produce specific, direct adaptive changes in hepatic carbohydrate-metabolizing enzymes.

13. Oral folic acid produces a specific stimulatory effect upon certain hepatic carbohydrate- and folate-metabolizing enzymes.

14. Oral ethanol inhibits certain specific hepatic enzymes (including two key enzymes involved in gluconeogenesis), and this is partially reversed by oral folic acid.

15. Ethanol, in vitro, significantly increases hepatic adenyl cyclase activity.

16. Clofibrate significantly decreases several hepatic glycolytic enzyme activities.

17. Intravenous glucagon, epinephrine, and insulin produce rapid changes in specific key hepatic glycolytic and gluconeogenetic enzyme activities.

18. Certain of the hormonal effects on hepatic enzyme activities (those produced by glucagon and epinephrine) can be mimicked by intravenous cyclic AMP and are unaffected by pre-treatment with inhibitors of protein synthesis.

19. Intravenous glucagon, epinephrine, and insulin produce rapid changes in hepatic and jejunal formiminotransferase activity.

20. Thyroxine and corticosterone appear to play largely permissive roles in the regulation of certain hepatic carbohydrate-metabolizing enzymes.

21. Oral folic acid was effective therapy in the treatment of one patient with hereditary fructose intolerance.

22. Oral folic acid has provided significant therapeutic benefit to five patients with hypoglycemia due to hepatic FDPase deficiency.

23. Jejunal fructosediphosphatase activity was low in eight out of thirteen patients with idiopathic reactive hypoglycemia.

24. Hepatic fructosediphosphatase activity was low in a preliminary study of six alcoholic cirrhotics.

25. Clofibrate therapy in conjunction with prota-caval shunting has provided dramatic improvements in two patients with glucose-6-phosphatase deficiency.

We have established the adaptive nature of hepatic enzymes, which, like intestinal enzymes, respond to numerous exogenous (diet, folic acid, ethanol) and endogenous (glucagon, insulin, epinephrine, thyroxine, corticosterone) factors.

E. Literature Cited

The following list of references (65 citations) constitute the published work derived from the studies done on the Metabolic Ward, USAMRNL, Denver, Colorado, under Army Contract DA-49-193-MD-2596 or in support thereof during the period July 1965 to April 1974.

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